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IMMUNOHISTOCHEMICAL EXPRESSION OF CD133 IN HIGH GRADE TRANSITIONAL CELL CARCINOMA OF THE URINARY BLADDER

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ABSTRACT

Background: Urothelial carcinoma (transitional cell carcinoma) of the urinary bladder, represents 90% of all primary tumors of this organ, and one of the most common ten malignancy in Iraq and worldwide. These tumors can range from low grade papillary neoplasms to less frequent more aggressive and invasive high grade tumors. More than 70% of superficial tumors recur, and about one-third of the patients have tumor progression, which can affect the quality of their life. Treatment failure, recurrence, and metastasis in bladder cancer are attributed to a subset of tumor cells expressing cancer stem cell (CSC) markers. CSCs have been isolated from leukemia and from a wide spectrum of solid tumors, including BC, using putative CSC markers such as OCT4, CD133, ALDH1, and CD44.

- **Aims of the study:**

This study aimed to provide a present outlook of urothelial carcinoma among Iraqi patients through the followings:
To assess the immunohistochemical (IHC) expression patterns of CD133
1. in high grade of urothelial carcinoma
2. in the epithelium of normal looking bladder tissue
3. To evaluate the expression of CD133 marker and its association with the Clinico- pathological parameters.

- **Materials and Methods:** cross sectional study which was carried out at the Department of Pathology and Forensic Medicine, at Al-Mustanssria college of medicine during the period from august 2017 to august 2018. A total number of 60 tissue samples were collected for the study, Patients were divided into two groups according to The pathological diagnosis of the bladder tissue

Group A: 30 case with high grade TCC of bladder.
Group B: (as a control group) 30case with apparently normal bladder tissue

- Tissue immunohistochemical analysis was applied to investigate the expression patterns of CSC markers CD133 in bladder cancer samples.
- **Results:** CD133, it was positive in 56.7% of specimens of group A, and16.6% in group B.

Regarding CD133 and high grade group, for associated inflammation and necrosis, 87.5% of those without inflammation showed positive CD133 marker result with a significant association (P=0.039) Concerning vascular invasion, 71.4% of patients with vascular invasion showed negative CD133 marker result with a significant association (P=0.003), In the area of muscular invasion, all patients who were free of muscular invasion showed positive CD133 marker result with a significant association (P=0.016) The majority of patients who had glandular differentiation and sequamous metaplasia showed negative CD133 marker result (84.6%) with a significant association (P=0.001) The highest prevalence of positive CD133 marker result was seen in patients without prostate involvement (66.7%) with a significant association (P=0.027).

- **Conclusions**

1-there was a significant expression of CSCs CD133 in transitional cell carcinoma.
2-there was an inverse correlation between the expression of cd133 and the pathological parameters of aggressiveness [muscular and vascular invasion and prostatic involvement] this may affirm that CD133 has a role in tumor initiation, but no role in tumor invasion and dissemination.

KEYWORDS: Urothelial carcinoma, immunohistochemical (IHC).

INTRODUCTION

Bladder cancer (BC) ranks as the most common neoplasm involving the urinary tract and the ninth most prevalent malignant tumor in the world.[1] It is the fifth most prevalent malignant tumor in Iraq.[2]

The most common type of bladder tumors diagnosed is transitional (urothelial) cell carcinoma (TCC); it constitutes more than 90% of bladder cancers. TCC can arise anywhere in the urinary tract, including the renal pelvis, ureter, bladder, and urethra, but it is usually found in the urinary bladder.[3]

Other types include sequamous cell carcinoma and adenocarcinomas.

Clinical and pathological data indicates there are at least 3 different phenotypes, as follows, exist in urothelial carcinoma: Low-grade proliferative lesions that develop into non–muscle-invasive tumors; these account for approximately 80% of bladder cancers, Highly proliferative invasive tumors with a propensity to metastasize, CIS, which can penetrate the lamina properia and eventually progress.[3]

The WHO classifies bladder cancers as low grade (grades 1 and 2) or high grade (grade 3).[4]

Tumors are also classified by growth patterns: papillary (70%), sessile or mixed (20%), and nodular (10%) Emerging evidence indicated a proportion of functionally different tumor cells termed cancer stem cells (CSCs) or tumor-initiating cells (TICs), which cause tumor growth and maintenance in BC. Stem cells can be sensitive to changes in their environment including toxic substances and smoking.[5,6] These changes within stem cells may potentially induce carcinogenesis by limiting their differentiating potential but expanding their proliferative potential. This process is directly connected with stem cell senescence in which DNA alterations play an important role.

Some unique characteristics of CSCs include an increased expression of telomerase and ATP-binding cascade transporters (ABC transporters) and evasion of apoptosis. Cancer stem cells have become the target in treating various cancers. It has to be tested what connections are between cancer stem cells and tumor initiating cells. Characteristic markers and proteins may help to identify bladder cancer stem cells and thus early stages of bladder cancer.[6,7]

CD133 (prominin 1)

Is a cell surface glycoproteinthat belongs to the pentaspan membrane family of proteins encoded by the PROM1 gene on ch.4. The biological role of CD133 is not yet known, but it is mainly localized to cellular protrusions and it has been proposed that CD133 may be involved in organizing cellular topology.[9]

CD133, at first, was introduced as a hematopoietic stem cell marker, and after that it was applied for isolation and characterization of CSCs from several solid malignant tumors. This evidence indicated that OCT4 and CD133 can shed light on the role of CSCs in BC tumorigenesis and eventually can be applied in the early diagnosis, in the determination of prognosis, in the prevention of tumor recurrence and in designing therapy.[10]

METHODS

This cross sectional study was carried out at the Department of Pathology and Forensic Medicine, at Al-Mustanssria college of medicine during the period from august 2017 to august 2018. Ethical approval for the study was obtained from the ethical committee of Al-Yarmook Teaching Hospital and forensic medicine directorate. This study was conducted on human bladder tissue specimens.

A total number of 60 tissue samples were collected for the study, 30 with high grade TCC were retrospective obtained from archives of histopathology unit of Al-Yarmook teaching hospital and private labs, with 30 prospective samples was obtained from the directorate of forensic medicine at Baghdad medical city. The patients' medical reports, with full clinico-pathological parameters were collected and reviewed. Patients were divided into two groups according to The pathological diagnosis of the bladder tissue by reviewing(haematoxylin and eosin stained slides): Group A: 30 case with high grade TCC of bladder.

All the samples of those group were formalin fixed paraffin embedded blocks. Group B: (as a control group) 30case with apparently normal bladder tissue taken by autopsy from forensic medicine directorate.

Immunohistochemical Staining

Expression patterns of CSC marker CD133 were explored using immuno-histochemical analysis applying the standard method. All sections were de-paraffinized at 60C for 20 minutes and dehydrated with graded alcohol. Tissue endogenous peroxides were blocked with 3% endogenous peroxidase in methanol for 20 minutes at room temperature. After washing 3 times, the antigen retrieval was performed by immersing the tissues in (pH=6.0) for CD133 for 10 minutes in an autoclave. The tissue sections were incubated with primary anti-CD133 antibody Elabscience biotechnology E-AB-16223 (dilution: 1/70) overnight at 40C. slides were then incubated with anti-rabbit/santa-cruz biotechnology as the secondary antibody for 30 minutes. Staining patterns were visualized by exposure to 3,3'-diaminobenzidine (DAB; Dako) solution for 15 minutes and counterstaining with hematoxylin (Dako). Finally, the slides were dehydrated in alcohol, cleared in xylene (Dako), and mounted for examination. In each run of the experiment, sequamous cell carcinoma of the lung as positive control for CD133 The omission of primary antibodies was used as negative control marker.

IHC evaluation of CD133: All samples were assessed without knowledge, and correlated with the parameters of the patient, it shows both cytoplasmic and membranous staining. The intensity was scored as: 0= negative, 1= weak, 2= moderate3= strong, Scoring of

staining, 0 = no staining +1 = <25% of +ve cell, +2 = 25 -50% of +ve cells, +3 = 51-75% of +ve cells, +4 > 75% positive cells.

Statistical Analysis: The data analyzed using Statistical Package for Social Sciences (SPSS) version 25. The data presented as mean, standard deviation and ranges. Categorical data presented by frequencies and percentages. Pearson's Chi–square test was used to assess statistical association between different associated variables. A level of P – value less than 0.05 was considered significant.

RESULTS

The distribution of study patients' groups by age is shown in table (1). Study patient's age was ranging from 40 to 84 years with a mean of 69.52 years and standard deviation (SD) of ± 11.17 years. The highest proportion of study patients in groups A was aged between 61 - 70 years (56.7%) while in group C, the highest proportion of was aged ≤ 50 years (63.3%).

Age Group (Years)	Group A n= 30 (%)	Group b n= 30 (%)
≤ 50	0 (0)	19 (63.3)
51 – 60	7 (23.3)	5 (16.7)
61 – 70	17 (56.7)	5 (16.7)
71 – 80	6 (20.0)	1 (3.3)
> 80	0 (0)	0 (0)

Scoring and intensity of CD133

In group A, CD133 marker scored (3) in 47% of the specimens of group A, scored (2) in 60% of specimens in group b.

Regarding intensity, moderate intensity was the most common intensity of CD133 marker in all study groups when it represented (58.8%, and 60% respectively).

CD133 Marker	Group A n= 17 (%)	Group b n= 5 (%)
Score 1	2 (11.8)	1 (20.0)
Score 2	6 (35.3)	3 (60.0)
Score 3	8 (47.0)	1 (20.0)
Score 4	1 (5.9)	0 (0)
Weak	1 (5.9)	1 (20.0)
Moderate	10 (58.8)	3 (60.0)
Strong	6 (35.3)	1 (20.0)

Association between CD133 marker result and certain clinic-pathological features of group A

The prevalence of positive CD133 marker result was increasing with aging and all patients aged > 70 years (100%) showed positive CD133 marker result with a significant association (P=0.001) between CD133 marker result and age.

About gender, all females showed positive CD133 marker result (100%) with significant association (P=0.032) between CD133 marker result and gender.

Regarding inflammation with necrosis, 87.5% of those without inflammation showed positive CD133 marker result with significant association (P=0.039) between CD133 marker result and inflammation with necrosis.

Concerning vascular invasion, 71.4% of patients with vascular invasion showed negative CD133 marker result with significant association (P=0.003) between CD133 marker result and vascular invasion.

In the area of muscular invasion, all patients who were free of muscular invasion showed positive CD133 marker result with a significant association(P=0.016) between CD133 marker result and muscular invasion.

The majority of patients who had glandular differentiation and squamous metaplasia showed negative CD133 marker result(84.6%) with a significant association(P=0.001) between CD133 marker result and glandular differentiation and squamous metaplasia.

The highest prevalence of positive CD133 marker result was seen in patients without prostate involvement (66.7%) with a significant association (P=0.027) between CD133 marker result and prostate involvement.

Table 3: Association between CD133 marker result and certain clinic-pathological features of group A.

Variable	CD133 Marker Result		Total n= 30	P – value
	Positive (%) n= 17	Negative (%) n= 13		
Age (Years)				
51 – 60	0 (0)	7 (100.0)	7 (23.3	**0.001**
61 – 70	11 (64.7)	6 (35.3)	17 (56.7	
71 - 80	6 (100.0)	0 (0)	6 (20.0	
Gender				
Male	12 (48.0)	13 (52.0)	25 (83.3	**0.032**
Female	5 (100.0)	0 (0)	5 (16.7	
Inflammation with necrosis				
Yes	10 (45.5)	12 (54.5)	22 (73.3	**0.039**
No	7 (87.5)	1 (12.5)	8 (26.7	
Vascular invasion				
Yes	4 (28.6)	10 (71.4)	14 (46.7	**0.003**
No	13 (81.3)	3 (18.8)	16 (53.3	
Muscular invasion				
Yes	11 (45.8)	13 (54.2)	24 (80.0	**0.016**
No	6 (100)	0 (0)	6 (20.0	
Glandular differentiation and squamous metaplasia				
Yes	2 (15.4)	11 (84.6)	13 (43.3	**0.001**
No	15 (88.2)	2 (11.8)	17 (56.7	
Prostate involvement				
Yes	1 (16.7)	5 (83.3)	6 (20.0	**0.027**
No	16 (66.7)	8 (33.3)	24 (80.0	

DISCUSSION

Age and gender

Current Study patient's age was ranging from 40 to 84 years with a mean standard deviation (SD) of 69.52 ± 11.17 years. Male predominance noticed In constituent to study conducted in Jordan (2008), as they found male predominance in their results (86% vs 14%) with male: female ratio 9:1 and the mean age of the patients was 60.6 (range 19-91) years (Matalka I et al, 2008). while higher than this results in regard to gender noticed in a study conducted in China (2007) involved 49 patients with bladder carcinoma, they noticed that there were 32 males (65.3%) and 17 females (34.7%) with male: female ratio was 1.3:1 and similarly in regard of age as was ranging from 44 to 80 years old (mean 63 years) (Xu k et al, 2007).

Also agreement notice with an Iranian study done in 2013, in which 138 cases were male (87%) and 21 cases were female (13%) with Male: Female ratio was 6.57. The mean age at the time of diagnosis was 64±12 years (range 23-87 years) (Keymoosi H et al, 2014) and Egyptian one in 2016, found that 76 male cases (90.5%) and eight female cases (9.5%), with a ratio of males-to-females of 10: 1. Age ranged between 42 and 82 years, with a mean age of 61.2±9.043 years (Asar A et al, 2017).

It was obvious that male predominance observed in studies mentioned above in addition to prevalence in elderly patients despite the differences noticed in results which might attributed to the difference in sample size and to the environmental factors caused bladder ca in each study.

Scoring and Intensity of markers in study groups

In regard to CD133, CD133 expression was found on the cytoplasm and the membrane of tumor cells[localized in microvilli and other plasma membrane protrusions], CD133 expression in a study in iran, they showed a variety of intensities, indicating moderate and strong intensity of staining in 79 (61%) and 36 (28%) cases, respectively, whereas negative and weak intensity was observed in a small proportion of tumor cells (6%) (Sedaghat S et al, 2017).

CSC marker result and study groups

Negative CD133 marker was significantly related to inflammation with necrosis (in group A) (P=0.039), to vascular invasion (P=0.003), to muscular invasion (P=0.016), to glandular differentiation and squamous metaplasia (P=0.001) and finally to prostate involvement (P=0.027).

In comparison to a study done in Iran 2017, they revealed a relative inverse correlation between the expression of CD133 with lamina propria invasion (P=0.051) and muscularis propria invasion (P=0.07) but differ in that no correlation found between CD133 expression and other clinicopathological parameters (Sedaghat S et al, 2017).

Furthermore, Significant associations were detected between CD133 expression and high-grade tumor

(*P*=0.041) and with the presence of muscle invasion (*P*=0.029) in an Egyptian study conducted in 2017 in constituent to the results of the present study (Wasfy RE et al, 2017).

- **CONCLUSION**

1-there was a significant expression of CSCs CD133 in transitional cell carcinoma.
2-there was an inverse correlation between the expression of cd133 and the pathological parameters of aggressiveness[muscular and vascular invasion and prostatic involvement] this may affirm that CD133 has a role in tumor initiation, but no role in tumor invasion and dissemination.

We recommend further prospective studies including larger sample size with follow up of patients to evaluate the relation of immuno-histochemical expression of CD133 to future outcome and chemotherapy responsiveness.

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